appreciates the extraordinary level of effort devoted by the Examiner and the Office to the application. As recognized by the Examiner, the application is significant in terms of its public policy implications, as well as its own scientific merits.

Claims 1-36 are present in this application. By this Amendment, Claims 1, 5, 6, 7, 9, 10, 13, 19, 23, 24, 25, 27, 28, 32, 33, 34, and 36 are amended, and new Claims 37-55 are added. Applicant respectfully requests that the Examiner reconsider the pending rejection, withdraw it in view of the amendments and additional information submitted in this Response, and allow the claims.

The application as originally drafted claimed various permutations of a chimeric embryo. More specifically, claims were drawn to chimeric embryos, and cell lines and animals derived from chimeric embryos. The Office has rejected the claims on five separate grounds:

- First, the Examiner has rejected Claims 1-9 and 13-36 under 35 U.S.C. § 101 as being directed to non-statutory subject matter, namely as falling within an exception to statutory subject matter as embracing a human being.
- Second, the Examiner rejected Claims 1-7, 10-25, and 28-34 under 35 U.S.C. § 102(b) as being anticipated by certain references disclosing: the introduction of human hematopoietic cells into sheep and mice *in utero*; mouse primordial germ cells and human fetal and embryo cell lines; and transgenic mice.
- Third, the Examiner has rejected Claims 1-9 and 19-36 under 35 U.S.C. § 103(a) as being obvious, in view of references disclosing: sheep/goat chimeras; chimeric sheep/goat pregnancies; chick/quail chimeric embryos; and chimeric mice.
- Fourth, the Examiner has rejected all claims under 35 U.S.C. § 112, first paragraph, based on the assertion that, in spite of the asserted obviousness and anticipation of

the invention by no less than nine separate references, the specification fails to contain an enabling disclosure, citing unpredictable outcomes in chimera formation, and the lack of fecundity of chimeric animals.

- Fifth, the Examiner has rejected all claims under 35 U.S.C. § 112, second paragraph as being vague and indefinite as to what would be considered a chimeric embryo in terms of viability.

Applicant respectfully submits that the variety, multiplicity, and inconsistency of the various rejections support the allowability of the claims as amended.

The subject matter of the appended claims is made by the intervention of man. The claimed subject matter is not naturally occurring and constitutes patentable subject matter under Section 101. The vast array of references cited by the Examiner establishes that the specification satisfies the requirements of Section 112. The techniques needed to make and use the claimed invention are well within the ordinary level of skill in the art as evidenced by the multiple references identified by the Examiner. In spite of the comprehensiveness of the art, no one has practiced, taught, or suggested the use of these well known and amply documented techniques to make the claimed invention. The Examiner has recognized the richness of the level of ordinary skill, yet, has identified no reference teaching or suggesting the claimed invention as a whole. Applicant respectfully submits that the claims, as amended, are patentable and respectfully requests that the claims be allowed.

I. The Application Claims Patentable Subject Matter (35 U.S.C. § 101)

Claims 1-9 and 13-36 are rejected under 35 U.S.C. § 101 as directed to nonstatutory subject matter. Specifically, the Examiner asserts that the claims are unpatentable because they "embrace a human being." In spite of the fact that they claim cell lines derived from both human and non-

human material, Claims 10-12 were not rejected as "embracing" a human being. The rejection has been applied against only the embryo claims. This rejection is respectfully traversed.

The rejection is improper for two reasons: (1) it is not a proper statutory requirement for patentability; and (2) the claimed subject matter is not a human being but rather, man-made chimeric cell lines, embryos and animals developing from them. Applicant respectfully submits that the Commissioner has no authority to reject the claims of the present invention--that are explicitly "made by man"--on the grounds that they "embrace a human being." Applicant respectfully submits that the above-outlined new claims and following remarks obviate the grounds for the rejection. In order to more fully describe the invention as claimed, Applicant hereby adds additional claims to **chimeric embryos containing human cells and nonhuman animals derived from such embryos**. Reconsideration and withdrawal of the rejection are respectfully requested.

As to the first point, the only issue is whether or not the claimed invention describes *statutory* subject matter. Nowhere does the statute restrict patentability based upon embracing a human being. To support this requirement, the Examiner relies upon the Commissioner's authority as construed by the U.S. Supreme Court in *Diamond v. Chakrabarty,* 447 U.S. 303 (1980) and interpreted by the Commissioner in Guidelines, 1077 OG 24 (Apr. 21, 1987). Neither provides a basis for the present rejection. Moreover, there is no *statutory* basis for imposing such a rejection under *Chakrabarty.*

The Examiner recognizes that the Court in *Chakrabarty* held that statutory subject matter shall "include anything under the sun that is made by man." (447 U.S. at 309, Office Action at p. 2). The claimed subject matter is not naturally occurring. It is not disputed by the Examiner that the claimed subject matter is "made by man." Applicant claims a chimeric embryo, a cell line, or animal derived from the chimeric embryo. A human being is not claimed.

The Examiner, recognizing that the claimed subject matter falls squarely within the scope of the Court's holding in *Chakrabarty*, that it is "made by man," injects the additional limitation that, although made by man, the invention cannot "embrace a human being." (Office Action at pp. 2-3). Although Applicant may be willing to concede that claims drawn to a human being "made by man," such as by artificial insemination, would be barred by the XIIIth Amendment, that principle provides no basis for the present rejection. The subject matter of none of the claims of the present invention is a human being.

There are three reasons why the Examiner's "embraces a human being" rejection is not proper. First, it has no statutory or other legal basis. Second, it contravenes established law defining what a "human being" is and the rights flowing from that status. Third, the rejection is inconsistent with established PTO practice of granting patents for a variety of inventions, many of which would "embrace a human being" equally or more than the claimed invention.

The Federal Circuit recently emphasized in *State Street Bank & Trust Co. v. Signature Financial Group*, 149 F.3d 1368 (Fed. Cir. 1998) that neither courts nor the Patent Office are authorized to embellish the statutory requirements for patentability. In *State Street*, the Federal Circuit confronted the so-called "mathematical algorithm" and "business method" exceptions to patentability. As the "embraces a human being" exception grafted by the Examiner in this case, these exceptions enjoyed no statutory sanction. Unlike the "embraces a human being" exception, they enjoyed prior judicial and Patent Office application in varying degrees.

Ironically, the PTO did not apply either extra-statutory exception to Signature's method of managing mutual funds. Unlike the present application, the claims were allowed and the patent issued. When Signature tried to license its patent, the prospective licensee brought a declaratory

judgement motion after negotiations broke down. The district court granted summary judgement of invalidity of the patent as not directed to statutory subject matter.

The Federal Circuit reversed, holding that neither the mathematical algorithm nor business method exception could preclude patentability, because neither had a basis in the statute. *State Street*, 149 F.3d at 1373, 1375-76:

> The repetitive use of the expansive term "any" in § 101 shows Congress's intent not to place any restrictions on the subject matter for which a patent may be obtained beyond those specifically recited in § 101. Indeed, the Supreme Court has acknowledged that Congress intended § 101 to extend to "anything under the sun that is made by man." *Diamond v. Chakrabarty*, 447 U.S. 303, 309, 100 S.Ct. 2204, 65 L.Ed.2d 144 (1980); *see also Diamond v. Diehr*, 450 U.S. 175, 182, 101 S. Ct. 1048, 67 L.Ed.2d 155 (1981).[3] Thus, it is improper to read limitations into § 101 on the subject matter that may be patented where the legislative history indicates that Congress clearly did not intend such limitations. *See Chakrabarty*, 447 U.S. at 308, 100 S.Ct. 2204 ("We have also cautioned that courts 'should not read into the patent laws limitations and conditions which the legislature has not expressed.' " (citations omitted)).
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> [3] The Committee Reports accompanying the 1952 Act inform us that Congress intended statutory subject matter to "include anything under the sun that is made by man." S. Rep. No. 82-1979 at 5 (1952); H.R.Rep. No. 82-1923 at 6 (1952).

Id. at 1373.

As the Federal Circuit has held so clearly in *State Street*, "any" invention "made by man" is patentable subject matter. It is for Congress--not the courts or the PTO--to set forth any limitations on patentable subject matter. Congress has not established any limitation based on subject matter that "embraces a human being." The Commissioner lacks the authority to impose one under Section 101. Whether or not the PTO believes Congress intended to bar patentability of inventions that

embrace a human being is not the issue. Congress has not done so expressly and the PTO has no authority to fill that gap.

Even were it possible to overlook the lack of a statutory basis for the new "human being" exception applied in this case--and it is not--such a standard is vague and hopelessly subjective. The Examiner has not specified how the claimed man-made chimeras "embrace a human being" or what features of a human are critical in doing so. "Chimeric embryos and animals containing human cells" can be considered to "embrace a human being" only in extreme cases and, even then, only in a subjective sense, none of which is the subject of this invention. All of the subject matter of the present claims is drawn to chimeras and, therefore, by definition to subject matter that is not human. The chimeras of the present invention are not naturally occurring and were unknown prior to the present invention.

Second, the Supreme Court has held that embryos, even those consisting exclusively of human cells, are not constitutionally protected as human beings (*see*, *Roe v. Wade*, 410 U.S. 113 (1973)). Congress--in spite of almost thirty years of vigorous public debate--has indicated no intention of altering this holding. That holding is mandatory authority and precludes the Examiner's finding that a single cell is sufficient to make a human being.

Embryos which are <u>not</u> exclusively human in origin, viz. the embryos of this invention, which contain human as well as animal cells, are not human beings. They do not fall under 1077 OG 24 (4/21/87). Utility of such chimeric embryos as experimental models in biomedical and developmental biological research was documented in the original application. These embodiments of the invention, which do not "embrace a human being," are appropriate subject matter for protection under 35 U.S.C. § 101. In order to clarify that certain claims exclude full term organisms

that the Examiner might consider "human," Applicant has amended the claims, to add additional claims to chimeric embryos not to exceed 10 days, 12 days, 14 days, 21 days, and 180 days to indicate that even if these embryos contain human cells, they could not possibly "embrace a human being" as delimited by prevailing statutory and case law.

Third, the present rejection is novel and unprecedented and this is the teeth of the Commissioner's established practice and procedure. As noted by the Examiner, mice and sheep have been engrafted with human bone marrow cells, and have been raised in laboratories as subjects of scientific investigations (Pixley et al., (1994) *Pathobiology* **62**, 238-44; Almeida-Porada et al., (1996) *Exp. Hematol.* **24(3)**, 482-7.

Pixley et al. established long term chimerism in normal mice transplanted *in utero* with human fetal hematopoietic stem cells. These human cells were injected into fetal mouse peritoneal cavities on days eleven through thirteen of gestation. These animals may develop and contain human cells in various organs. This engraftment of human cells into mouse fetuses does not now qualify the mouse as a human being, nor does it create a human being. The Office has never held that it does prior to the present invention and has regularly granted patents on such inventions.

Almeida-Porada et al. describes the transplantation *in utero* of preimmune fetal sheep with human hematapoietic stem cells which result in a long term chimerism. These experiments reported the long term persistence of human cells in the human/sheep zenograft model. As with the above, the sheep, although containing human cells, are not considered human beings.

While Applicant disputes the Examiner's claim that these studies represent prior art with respect to the present invention (see below), it is clear that these organisms represent "animals containing human cells." They are not constitutionally protected as human beings. Because specific

utility of non-human animals containing human cells, constructed by the methods of this invention, was documented in the original application, such organisms do not fall under 1077 OG 24 (4/21/87).

Applicant respectfully submits that a proportion of human cells in an organism does not make that organism a human being. In addition, the original application does not include any claims to a human being, but only contains claims to a chimera or an animal or cell line derived from the chimera. The fact that a chimera has a human cellular component cannot exclude it from patentability, any more than the many patents that share that feature and have been awarded by the PTO. Subject matter consisting of, or derived from, human cells in non-human animal systems has been, and continues to be, granted patents in the area of biotechnology.

II. The Claims Define Patentable Subject Matter

Each of the claims has been rejected under Sections 102 and/or 103 over a combination of one or more of nine varied references. If, as the Examiner contends, the claimed invention was anticipated and/or obviated by one or more of these references, the multiplicity and complexity of combinations of them would be unnecessary. In fact, none show each element of the claimed subject matter, either alone or in combination. Perhaps more important, the unpredictability of the science--one of the benefits of the present invention--belies the conclusion that the invention as a whole would have been known by or obvious to one of ordinary skill in the art.

A. The Claims are Patentable over Zanjani et al. or Almeida-Porada et al. or Pixley et al.

Claims 1-7, and 13-25 are rejected under 35 U.S.C. § 102(b) as being anticipated by Zanjani et al. (1996) *Int. J. Hematol.* **63**, 179-192 or Almeida-Porada et al. (1996) *Experimental Hematol.* **24**, 482-487. Claims 1-7, and 13-25 and 28-34 are rejected under 35 U.S.C. § 102(b) as being

anticipated by Pixley et al. (1994) Pathobiol. **62**, 238-244. These rejections are respectfully traversed.

The Examiner states that the invention was anticipated by the description by Zanjani et al. (1996) and Almeida-Porada et al. (1996) of the introduction of human hematopoietic cells into sheep *in utero*, and by Pixley et al. (1994) of the introduction of human hematopoietic cells into mice *in utero*. Zanjani and his associates (Flake, A. W., and Zanjani, E. D. (1993) *In utero* Transplantation of Hematopoietic Stem Cells. *Crit. Rev. Oncol. Hematol.* **15,** 35-48; Pixley et al. (1994); Zanjani et al. (1996); Almeida-Porada et al. (1996); Pixley, J. S., Zanjani, E. D., Shaft, D. M., Porada, C., and Mackintosh, F. R. (1998). Prolonged Hematopoietic Chimerism in Normal Mice Transplanted *in utero* with Human Hematopoietic Stem Cells. *Pathobiology* **66,** 230-9) conducted late embryo grafting experiments to produce hematopoietic organisms, i.e., mixtures of blood forming cells in an organism (a sheep or a mouse) that is unambiguously of one species.

The present invention, in contrast, claims an embryo, cell line, or embryo developing into an organism that is a true chimera. The organisms produced by Zanjani et al., Almeida-Porada et al., and Pixley et al. do not have the composite morphology and multi-tissue chimerism of the early embryo chimeras described by Fehilly et al. (1984) and Meinecke-Tillmann and Meinecke (1984). The work of the Zanjani et al., Almeida-Porada et al., or Pixley et al. groups represents xenograft models, not chimeric embryos. None anticipates the claimed invention.

The present invention describes chimeric embryos containing human cells, where aggregation of **totipotent** cells (i.e., blastomeres or ES cells) of two or more species is performed. This is entirely different, and leads to different developmental outcomes, than the engraftment of **multipotent** stem cells during fetal stages.

Applicant respectfully submits that Zanjani et al., Almeida-Porada et al., or Pixley et al. fail to disclose the subject matter of the claimed invention. Reconsideration and withdrawal of these rejections is respectfully requested.

B. The Claims are Patentable over Cheng et al., or Catalog of Cell Lines and Hybridomas.

Claims 10-12 are rejected under 35 U.S.C. § 102(b) as being anticipated by Cheng et al. (1994) *Develop.* **120**, 3145-3153. Claims 10-12 are also rejected under 35 U.S.C. § 102(b) as being anticipated by Catalog of Cell Lines and Hybridomas, 7th ed., American Type Culture Collection (ATCC), Rockville, MD. 20852-1776, 1992, entry HTB 157, HTB 158, and HTB 160, page 271. The Examiner states that the description of mouse primordial germ cells by Cheng et al. anticipates Claims 10-12 of the invention, as does the existence of human cell lines in the American Type Culture Collection (Office Action at pp. 11-12). These rejections are respectfully traversed.

Cells derived from chimeras are known to differ in immunological properties from equivalent cells in non-chimeric animals. One of ordinary skill in the art would expect that this would also likely pertain to cell lines derived from chimeras. Much could be learned from comparison of the properties of cell lines derived from the human/non-human chimeras of the present invention with cell lines derived from non-chimeric embryos or organisms, such as those described by Cheng et al.

The Examiner also states that "the animals that are prepared from the chimeras may not harbor any transgenes if the germ cell from which the animals derive did not harbor a transgene" (Office Action at p. 12). Applicant respectfully submits that this is incorrect. With the exception of Claims 16-18, which refer to decedents of chimeric animals, all animals referred to in the claims

are "developed" from chimeric embryos, which means that they are not the result of reproductive breeding. The presence or absence of transgenes in germ cells is irrelevant.

Applicant respectfully submits that Cheng et al. or American Type Culture Collection fail to disclose the subject matter of the claimed invention. Reconsideration and withdrawal of these rejections is respectfully requested.

C. The Claims are Patentable over Bradley et al.

Claims 13-18 are rejected under 35 U.S.C. § 102(b) as being anticipated by Bradley et al. (1992) *Bio/Technology* **10**, 534-539. This rejection is respectfully traversed.

The Examiner states that the description of transgenic mice by Bradley et al. anticipates Claims 13-18 of the invention. The animals of Claims 13-15, however, are **developed from chimeric embryos**. Fehilly et al. (1984) and Meinecke-Tillmann and Meinecke (1984) disclose that the form, appearance, and biology of any such animals would be very unlike the non-chimeric transgenic animals described by Bradley et al., which in contrast would be immediately identifiable as to species. The Examiner has cited no evidence to the contrary. With regard to the progeny or decedents of chimeric animals, claimed in Claims 16-18, although these would themselves be non-chimeric, their immunological and reproductive properties inevitably would be altered by the process of being bred from chimeras, as described above, and therefore would be biologically distinct from progeny or decedents of non-chimeric animals.

Applicant respectfully submits that Bradley et al. does not disclose the subject matter of the claimed invention. Reconsideration and withdrawal of these rejections are respectfully requested.

D. The Claims are Patentable over Gustafson et al.

Claims 1-7, 19-25 and 28-34 are rejected under 35 U.S.C. § 103 as being unpatentable over Gustafson et al. (1993) *J. Reprod. Fert.* **99**, 267-273. This rejection is respectfully traversed.

The Examiner cites Gustafson et al. as disclosing the ability of sheep-goat chimeras to gestate sheep-goat hybrid conceptuses and states that producing human/non-human chimeras to perform similar studies would therefore have been obvious. Interspecific chimeric mammalian embryos have been described at least since 1973 (Stern, M. S. (1973) Chimaeras Obtained by Aggregation of Mouse Eggs with Rat Eggs. *Nature* **243**, 472-3), interspecific-*intra*generic chimeric mammalian organisms at least since 1982 (Rossant, J., Croy, B. A., Chapman, V. M., Siracusa, L., and Clark, D. A. (1982) Interspecific Chimeras in Mammals: A New Experimental System. *J. Anim. Sci.* **55**, 1241-8), and interspecific-intergeneric chimeric mammalian organisms at least since 1984 (Fehilly, C. B., Willadsen, S. M., and Tucker, E. M. (1984) Interspecific Chimaerism Between Sheep and Goat. *Nature* **307**, 634-6; Meinecke-Tillmann, S., and Meinecke, B. (1984) Experimental Chimaeras--Removal of Reproductive Barrier Between Sheep and Goat. *Nature* **307**, 637-8). Yet, none of this substantial body of published research, or subsequent teachings, including Gustafson et al. (1993) taught or suggested the claimed invention: chimeric embryos and animals **containing human cells**; or the specific beneficial uses recited in the specification for directly studying **human** developmental biology, physiology, and toxicology.

Paradoxically, the Examiner argues that the production of human/non-human chimeric embryos would be "unpredictable," (Office Action at pp. 4-6) and argues here that "for achieving an embryo, which can be as small as one cell, Gustafson et al. provides sufficient teachings and motivation," (Office Action at p. 13). Leaving aside the fact that--by definition--a chimeric embryo

cannot be "as small as one cell" (it must be at least two cells), the present invention cannot simultaneously be both unpredictable and obvious. As the references cited by the Examiner establish, the area of developmental biology is unpredictable, making the utility of the present invention so powerful. It is for this reason, among others, that the claimed subject matter is not obvious.

Applicant respectfully submits that Gustafson et al. does not disclose the subject matter of Claims 1-7, 19-25 and 28-34. Reconsideration and withdrawal of these rejections is respectfully requested.

E. The Claims are Patentable over Watanabe et al., in view of Robertson et al.

Claims 1-7, 19-25 and 28-34 are rejected under 35 U.S.C. § 103 as being unpatentable over Watanabe et al. (1992) *Develop.* **114**, 331-338 in view of Robertson et al.(1986) *Nature* **323**, 445-448. The Examiner further argues that Claims 1, 8, 9, 19, 25-28, 35 and 36 are rendered obvious by previous teachings on how to identify the location and developmental potential of donor cells in chick-quail (Watanabe et al. (1992)) and mouse-mouse (Robertson et al. (1986)) chimeras. This rejection is respectfully traversed.

Neither Watanabe et al., either alone or in combination, or Robertson et al. disclose the present invention. Niether discloses chimeric embryos and animals **containing human cells**, let alone the specific uses in directly studying **human** developmental biology, physiology, and toxicology described in the present specification. Lineage tracing methodologies are described in the specification, that are similar to Robertson et al., Watanabe et al., and many investigators before and since. No claim is directed to those methodologies. Rather the specification discloses some of

the ways in which the claimed subject matter could be used. The obviousness that the Examiner argues is based on hindsight afforded only by the present application.

Applicant respectfully submits that Watanabe et al. and Robertson et al. do not disclose the subject matter of Claims 1, 8, 9, 19, 25-28 and 35 and 36. Reconsideration and withdrawal of these rejections are respectfully requested.

The ability to construct chimeras has only recently become possible due to advances in technology. In summary, none of the myriad references cited by the Examiner, either alone or in combination, anticipate or obviate the claimed invention. The patentability of the subject matter of the claimed invention is amply evidenced by the numerous references by the Examiner that ample teachings were available of various techniques disclosed in the specification to make and use the invention. Nonetheless, the field of the invention is so complex and unpredictable that one of ordinary skill in the art would not have known, or considered obvious, the claimed subject matter:

- "In regard to the nature of interspecies chimeras, (as are the claimed human/non-human animal chimeric embryos, animals developed from the embryos, and cell lines developed from the chimeric embryos), the art at the time of filing indicates that among problems with unpredictable outcomes are the loss of one species contribution over another, and that such loss is without *a priori* prediction of which of the parental species are lost, and a lack of fecundity of chimeric animals that may be formed under particular circumstances." (Office Action at p. 4).

- "Such unpredictability is evidenced by consideration of the sheep-goat chimeras reported by Fehilly et al. (1984) Nature 307, 634-638 (referenced in the instant specification at page 2, first paragraph)". (Office Action at pp. 4-5).

- "Thus, these teachings indicate not only that ES cells have not been prepared across species despite significant effort by the artisan, but also indicate that the animals from which ES cells are to be prepared must be considered and represent a factor contributing to the unpredictability of the establishment and use of ES cells." (Office Action at p. 9).

- "This problem is exacerbated when one considers that the instantly claimed invention is drawn to humans and other animal species in which well characterized, genetically uniform, strains are not available and unlikely to be generated given that many animals in general, and primates in particular, have long generation times, and limited abilities to be inbred in the absence of the large numbers of animals that are required to be culled to generate viable strains." (Office Action at p. 9).

- "Therefore, given the unpredictable nature of the claimed invention, the artisan would have been required to have exercised undue experimentation in the elaboration of which paticular combinations of donor species that would result a human/non-human animal chimera for each particular combination." (Office Action at p. 9).

- "In addition, given the lack of guidance and unpredictability in obtaining ES cells for the breadth of the claims as supported by the teachings of the art at the time of filing that the establishment and culture of ES cells was unpredictable, the production of chimeric human/non-human animal chimera from ES cells is also not enabled." (Office Action at p. 9).

III. The Claims Satisfy 35 U.S.C. § 112, First Paragraph

Claims 1-36 were rejected under 35 USC § 112, First Paragraph for lack of adequate enabling disclosure. The Examiner has taken the position that the specification fails to provide an enabling disclosure for how to make and use the claimed invention. This rejection is respectfully traversed. Applicant respectfully submits that the claim amendments and remarks below obviate the grounds for the rejection. Reconsideration and withdrawal of the rejection are respectfully requested.

Applicant's specification describes three specific technologies for making interspecific embryo chimeras, with citations to the published literature. The Examiner has cited dozens of references, establishing that the techniques are not only well known in the published literature, but readily apprehended and used by researchers in the art for a wide variety of investigations.

The first technology described in the specification is divided into three techniques, which are each described with appropriate citations. The first technology described in the specification aggregates cells derived from the early embryos of two or more different animal species or strains. These cells, will, under favorable circumstances, remain attached to one another and cooperate to form a more developed embryo, then a juvenile, and ultimately an adult organism exhibiting features in common with, and different from, individuals of the species or strains from which the embryo cells originated. Fehilly, C. B., Willadsen, S. M., and Tucker, E. M. (1984) Interspecific Chimaerism Between Sheep and Goat. *Nature* **307**, 634-6 and Meinecke-Tillmann, S., and Meinecke, B. (1984) Experimental Chimaeras--Removal of Reproductive Barrier Between Sheep and Goat. *Nature* **307**, 637-8 reported the experimental formation of sheep-goat chimeric embryos, and birth of chimeric "geeps" after transplantation to sheep or goat mothers using this technique.

Fehilly et al. described three individual methods to create chimeric embryos which further define, and are a part of, the first technology described above. The three methods were employed in an attempt to create sheep-goat chimeras. In the first method, single blastomeres from four-cell goat embryos were combined with single blastomeres from four-cell sheep embryos, or with single blastomeres from eight-cell sheep embryos in evacuated zonae pellucida as described in S.M. Willadsen (1979) *Nature*, **277**, 298-300.

In the second method of the first technology, interspecific embryos were produced by either surrounding an eight-cell goat embryo from which the zona pellucida had been removed with the dissociated blastomeres of three eight-cell sheep embryos or by surrounding a similarly denuded eight-cell sheep embryo with the separated blastomeres of three eight-cell goat embryos.

The embryos resulting from the first two methods discussed by Fehilly et al. were embedded in agar and cultured in ligated sheep oviducts for four or five days, depending upon the particular experiment. Those embryos which developed into normally organized chimeric blastocysts, were then transferred to recipient ewes or recipient goats.

In the third method of the first technology, the inner cell mass and polar trophectoderm from day eight goat blastocysts were inserted into day eight sheep blastocysts, and vice-versa, by the technique described by R.L. Gardner (1968) *Nature*, **220**, 596-597. The blastocysts were then transferred to sheep or goat recipients.

Fehilly et al.'s experiments demonstrate that sheep and goat blastomeres can form chimeric blastocysts and that such inter-species blastocysts are viable and may give rise to animals that are sheep-goat chimeras. The experiments also demonstrate that a goat fetus can develop to term in a sheep, and a sheep fetus can develop to term in a goat.

Meinecke-Tillmann and Meinecke (1984) used the first technology to create interspecific sheep-goat chimeric embryos. The embryos were created by combining one sheep four-cell blastomere with two goat eight-cell blastomeres, or by combining two sheep eight-cell blastomeres with two goat eight-cell blastomeres, in a common pig zona pellucida. Micromanipulation of the blastomeres was performed as described by S. Meinecke-Tillmann and B. Meinecke (1983) *Zentbl. Vet. Med.*, **30**, 146-153. The embryos that continued to develop were transferred to the oviducts of an intermediate recipient and embryos that reached the blastocyst stage were then transferred to the uterine horns of the final sheep recipients.

The second technique discussed in Applicant's specification for generating chimeric embryos is through the use of embryonic stem (ES) cells. ES cells are undifferentiated, immortal cells. They

are derived from the inner cell mass (ICM) of preimplantation mammalian embryos, by culturing these embryo cells under defined conditions. These cells are totipotent, i.e., capable of differentiating into derivatives of all three of the basic embryonic germ layers, from which all cell types ultimately develop. Martin (1981) *Proc. Nat. Acad. Sci. USA* **78**, 7634-7638, described the establishment of an ES cell line from the mouse embryo. Thomson et al. (1995) *Proc. Nat. Acad. Sci. USA* **92**, 7844-7848, described the isolation of an ES cell line from the embryo of the rhesus monkey. ES cells have the ability to remain undifferentiated and proliferate indefinitely *in vitro* while maintaining the potential to differentiate into derivatives of all three embryonic germ layers. Thomson et al. suggests that the use of human ES cells would offer "exciting new possibilities for transplantation medicine." (Thomson, et al. (1995) *Proc. Nat. Acad. Sci. USA* **92**, at 7848.) When combined with normal preimplantation embryos of the same or different strain from which they were derived, ES cells participate in normal development, potentially contributing cells to the tissues of the resulting animal (Bradley, et. al. (1984) *Nature*, **309**, 255-256).

The third technique discussed in Applicant's specification for generating chimeric embryos is through the use of "early passage" ES cells, i.e., cells that have been permitted only a few divisions in culture after the ES cell line is established, or certain ES cell subclones that retain totipotency at later passages. These cells are aggregated with defective embryos genetically incapable of advancing beyond the early stages of development, but which provide components that mediate implantation of the chimeric cell aggregates. Using this technique Nagy et al. (1993) *Proc. Nat. Acad. Sci. USA* **90**, 8424-8428 generated viable, normal, fertile mice, which were completely ES-cell derived. The technique is based on the aggregation of ES cells with developmentally compromised tetraploid

embryos. In such chimeras the tetraploid is selected against and ES cells differentiate normally, to form viable embryos.

All early mammalian embryos, including human embryos, undergo the same initial developmental steps. All go through a *two cell, four cell*, and *eight cell* stage, and all are initially surrounded by an extracellular layer known the *zona pellucida*. All form a hollow *blastula*, containing an *inner cell mass*. The inner cell mass further develops into two or more layers of cells known as the germinal layers. The germinal layers, the ectoderm, the mesoderm, and the endoderm, give rise to the various cell types that make up the adult animal. (An Introduction to Embryology, Fourth Edition (1975), Balinsky, B.I., W.B. Saunders Company, Philadelphia PA; Molecular Biology of the Cell, Second Edition (1989), Alberts, B. et al., Garland Publishing, Inc., New York, NY). Applicant respectfully submits that methods to create chimeric embryos were adequately disclosed and enabled the present invention. See Declaration of Stuart Newman, attached as Exhibit A.

Methods for making *intraspecific* mammalian chimeras (e.g., tetraparental mice) have been disclosed in the literature since the 1960's (Mintz, B., and Baker, W. W. (1967). Normal Mammalian Muscle Differentiation and Gene Control of Isocitrate Dehydrogenase Synthesis. *Proc Natl Acad Sci USA* **58**, 592-8), and are standard art in developmental biology. These methods were well known in the field of mammalian embryology.

The first reported *interspecific* chimeras between mouse and rat (Stern, M. S. (1973) Chimaeras Obtained by Aggregation of Mouse Eggs with Rat Eggs. *Nature* **243**, 472-3) were made by the same technique. The first reported interspecific chimeras that yielded viable animals between two species of mouse (Rossant, J., Croy, B. A., Chapman, V. M., Siracusa, L., and Clark, D. A.

(1982) Interspecific Chimeras in Mammals: A New Experimental System. *J. Anim. Sci.* **55**, 1241-8) also used the techniques originally developed for intraspecific mammalian chimeras. These interspecific chimeras in mammals were made by mixing embryonic cells from two species of mouse.

Fehilly et al. utilized the blastocyst injection technique developed by Gardner for the production of mouse-mouse chimeras (Gardner, R. L. (1968) Mouse Chimeras Obtained by the Injection of Cells into the Blastocyst. *Nature* **220**, 596-7), without modification, to produce sheep-goat chimeric embryos. Fehilly et al. used the blastocyst injection technique to produce sheep/goat chimeras by embryo manipulation and the use of interspecific chimerism to allow successful interspecific embryo transplantation in sheep and goats.

Technical improvements that have been made in handling early mammalian embryos since the original mouse-mouse and mouse-rat chimeras were reported, have been employed for chimera production. For example, Willadsen devised a method for culturing early sheep embryos in evacuated zonae pellucida and agar (Willadsen, S. M. (1979) A Method for Culture of Micromanipulated Sheep Embryos and its Use to Produce Monozygotic Twins. *Nature* **277**, 298-300).

Although this technique was not specifically developed for the production of chimeras, it has become widely used in animal reproductive science. Without further modification, it proved suitable for the production of viable sheep-goat chimeras in the aforementioned study of Fehilly et al., who used it as an alternative to the Gardner mouse technique, with equal success. In the same year, Meinecke-Tillmann and Meinecke (1984) produced sheep-goat chimeras, using a variation of the Willadsen technique, referred to above, employing a *pig* zona pellucida, e.g., an extracellular matrix

different from either of the parental species of the embryo cells. Fehilly et al. establishes that this technical variation, while contributing to an effective methodology for chimera production, was not required.

These studies establish that it was known in the published literature that the technology for producing chimeric mammalian embryos is "robust," i.e., insensitive to variations in procedure, species origin of the cells, or species origin of the zona pellucida. Techniques developed for mouse embryo culture, sheep embryo culture, mouse-mouse, and mouse-rat chimeras, have all proved successful for sheep-goat chimera production, and even the biological materials of the culture environment may be derived from a third species, with no detriment to the outcome. Applicant respectfully submits that there is absolutely no indication from the scientific literature that use of a different mammalian species, such as the human, would require "undue experimentation" for the design of a protocol for producing chimeras. Rather, the references cited by the Examiner reflect the utility of these techniques across species. Applicant therefore respectfully submits that using the cited references, the present invention is adequately enabled.

There is no indication from the scientific literature that a mixture of embryo cells from *three* species (e.g., human, and two non-human mammalian species), as specified in some embodiments of the invention, would immediately die, i.e., become non-viable, and therefore be unavailable as a research tool for developmental biologists and immunologists. Rather, the indication from existing knowledge is that the three cell types will cooperate with one another in building embryonic tissues and organs, and the extent to which this cooperation succeeds or fails in particular instances will provide important scientific information, one important feature of the utility of the invention.

Using the description of the Examiner, it is precisely the "human/human, human animal 1, human/animal 2, animal 1/animal 1, animal 1/animal 2, animal 2/animal 2, and human/animal 1/animal 2" interactions that can be productively studied by existing developmental biological and immunological techniques, but for which there is no currently existing experimental object in which these interactions can be studied. Existing methodologies of transplantation medicine, and protocols in cardiovascular physiology, could be brought to bear on a tri-species chimeric animal produced from the subject matter of the present invention, to assay the suitability of the tissues and organs for human transplantation.

The Examiner cites "unpredictable outcomes" as a basis for rejecting the claims. All biotechnological procedures inherently lead to unpredictable outcomes. Genetic and other uncontrolled biological variability of organisms necessarily make outcomes unpredictable. This has not disqualified previously patented biological inventions, and should not disqualify the present invention. Moreover, it is not necessary or appropriate to require predictability as a condition of patentability. The degree of predictability of the present invention is consistent with the claimed utility of the subject matter of the invention.

For example, U.S. Patent No. 4,736,866, "Transgenic Non-Human Animals," contains, among others, a claim to: "A transgenic non-human mammal all of whose germ cells and somatic cells contain a recombinant activated oncogene sequence introduced into said mammal, or an ancestor of said mammal, at an embryonic stage." The species of mammal is not specified, nor is the oncogene or the exact location of the oncogene. The invention has thousands, if not millions of embodiments with unpredictable outcomes.

It is not clear why the Examiner finds that the "unpredictable outcomes" of the present invention disqualifying. The level of predictability, reproducibility, or "quality control" requested by the Examiner is not appropriate and is not required for other similar inventions. The degree of predictability is a function of the claimed subject matter. It is particularly inappropriate to require a high degree of predictability in the present instance since, as described in the following paragraph, the variability of outcome is one of the useful aspects of our invention as a research tool.

The Examiner refers to studies which document variations in the degree of chimerism obtained in sheep-goat studies, which depend on the ratio of cells of the different parental types in the original embryo, the degree of similarity of the genotype of the embryo to the gestational environment, and other uncontrolled factors. One major set of uses specified for the present invention is in developing suitable models for developmental biological and immunological research (Application at pp. 18-21). The present invention will facilitate answering scientifically and medically important questions concerning the survival of cells of one species in the environment of cells of another species, and the immunological tolerance of the gestational environment of nonhuman species for developing tissues and organs that contain human cells.

There is no *a priori* way to determine what proportion of the embryo will ultimately be due to each species' cells. After development has progressed, however, this would be easy to ascertain using species-specific molecular markers or "reporter" genes, as described in the application (Application at p. 8).

In developmental biological research there is no requirement for implantation of the chimeric embryo to obtain useful information on early development and interspecies compatibility. Much work is done *in vitro*. The survival rates of implanted chimeric embryos will be irrelevant for certain

embodiments of the invention, and the invention provides a unique research tool in other embodiments. The claimed invention is not limited to a specific degree or range of chimerism or chimeric lifetime. All such variations are within the subject matter of the invention.

In this regard the inappropriateness of the additional requirement of predictability of outcome is stark. The degree of predictability necessary to eliminate undue experimentation is a function of the claimed subject matter and its utility. Experimentation need not be eliminated; it must not be *undue*. With respect to the claimed subject matter, some degree of unpredictability is affirmatively desirable.

Applicant has not claimed, for example, either a specific product or a product by process useful for treatment of a specific disease. Carrying this example forward, had Applicant claimed a product, namely an organ, such as a heart developed from a human/baboon chimeric embryo, suitable for transplantation into a human heart disease patient and designed to minimize graft-host rejection or tailored to certain performance levels, Applicant would agree with the Examiner that the present level of unpredictability would result in undue experimentation. The art in general, and the claimed subject matter in particular, has not advanced to the point of eliminating undue experimentation for such a claim. That is an invention that awaits other researchers.

Applicant, however, has made no such claim. The degree of description required is measured relative to the claims Applicant has made and utility specified by the Applicant. Applicant has specified among other things, a medical research model useful in developmental biology, and perhaps to make the further invention specified above. That utility affirmatively employs the high degree of complexity and variability from interspecific cellular interactions. Far from establishing

the specification is not enabling, that degree of unpredictability is an essential component of the utility of the claimed invention.

The Examiner states on page 4 of the Office Action that Applicant fails to provide an enabling disclosure for "viable" chimeric embryos. The Examiner further states that "viable chimeric embryos may be considered to be those that give rise to independent animals and/or human beings." But this is not the definition of the term "viable" in the embryological literature. Viable means "not dead" rather than "full term." For example, in Loi P, Ledda S, Fulka J Jr, Cappai P, Moor RM (1998) Development of Parthenogenetic and Cloned Ovine Embryos: Effect of Activation Protocols. *Biol. Reprod.* **58**, 1177-87, it is stated:

> Over 70% of parthenogenotes were **viable** on Day 21 of pregnancy but dead by Day 25... Moreover, cloned embryos developed to blastocyst stage in higher percentage after 6-DMAP treatment (83% vs. 25%). We conclude that ionomycin followed by 6-DMAP incubation yields high percentages of diploid parthenogenetic embryos that develop to Day 25 before dying. [Emphasis added].

"Viability" of embryos refers to continued development for a period of time sufficient for them to be studied, not development to full term.

Behr et al., (Behr B, Pool TB, Milki AA, Moore D, Gebhardt J, Dasig D (1999) Preliminary Clinical Experience with Human Blastocyst Development *in vitro* Without Co-culture. *Hum. Reprod.* **14**, 454-7), report:

> The embryos were cryopreserved using a standard protocol with serial addition of glycerol. Embryos reaching the blastocyst stage after more than 120 h of culture were not included. To date, 16 patients have each had up to three thawed blastocysts transferred, out of whom seven became pregnant. This report demonstrates that a simple system of sequential culture generated acceptable, **viable** blastocyst

> development (54%) with supernumerary embryos, without the use of feeder cells, conditioned medium or whole serum. [Emphasis added.]

Here again, "viability" refers to development at least a specified post-treatment stage, not to full term, or even pregnancy. Applicant respectfully submits that the chimeric embryos of the present invention would be "viable" by these generally accepted definitions, which reflect how the term "viable" would be understood by persons of ordinary skill in the art.

The Examiner also relies upon the possible "lack of fecundity of chimeric animals" as a basis for rejecting the claims. Fecundity was never a necessary characteristic of the present invention. Even if fertile, chimeric animals would never breed true because by definition their germ lines would be mosaics of the two originating species. At best, a mating between chimeric animals could give rise to offspring that were of one, or the other, originating species. Although some patented organisms are self-propagating, most compositions of matter are not. The inability to self-propagate is not a requirement for patentability under 35 U.S.C. § 101.

The invention claims decedents of chimeric animals (Claims 16, 17 and 18), not because these descendants would themselves be chimeras, but because the germ cells of either species that were produced within such chimeras would reasonably be altered because of their origin within a chimeric organism (Sumantri C, Boediono A, Ooe M, Murakami M, Saha S, Suzuki T. (1997) The Effect of Sperm-Oocyte Incubation Time on In Vitro Embryo Development Using Sperm from a Tetraparental Chimeric Bull. *Anim. Reprod. Sci.* **48**, 187-95). These organisms and their decedents would be important tools for developmental biological and reproductive biological research.

The Examiner further suggests that the human/non-human animal chimeras that Applicant claims involve a more diverse set of organisms than "the relatively limited range (sea urchin,

amphibian, avian, and mammal)" and that it would require undue experimentation to generate chimeras between such diverse organisms (Office Action at pp. 6-7). The range listed by the Examiner includes a non-vertebrate phylum (echinoderms) and two non-mammalian vertebrate classes (amphibia, aves). All the embodiments of Applicant's invention listed in the specification are chimeras among mammalian species (humans, non-human primates, mice, swine), a much narrower and more closely related set than that listed by the Examiner. All the techniques specified by Applicant were from mammalian embryology, and the feasibility of producing human/non-human animal chimeras is justified by the success of these techniques using mammalian species more distantly related than humans and chimpanzees: i.e., rats and mice, and sheep and goats. Applicant's invention relates to chimeras of human cells and cells of non-human **mammals**. Applicant has amended Claim 1 to read "**wherein said second animal species is one or more non-human mammals.**"

The Examiner questions the use of ES cells in forming the chimeric embryos of the invention, stating that "[a]t the time of filing, the art regarded as unpredictable the obtaining of ES cells that would contribute to the germ line of the resultant animal" (Office Action at p. 7). The production of self-propagating chimeras by the embryo fusion techniques of the specification is biologically impossible for the reasons discussed above. The contribution of the ES cells to the germ line of the resultant animal is not a feature of the invention. Baribault et al., (1989) *Mol. Biol. Med.* **6**, 481-492, on the difficulties of assuring that ES cells will lead to germ line chimerism, is not relevant to the present invention.

The Examiner states, moreover, that germ line chimerism is "a hallmark of an ES cell line." Applicant respectfully submits that this is not the defining characteristic of such a cell line, according

to the scientific literature. For example, Wheeler (1994) describes the production and validation of swine ES cells with no recourse to tests of germ line chimerism (Wheeler MB (1994) Development and Validation of Swine Embryonic Stem Cells: A Review. *Reprod. Fertil. Dev.* **6**, 563-8). Wheeler reported the establishment of procine embryonic stem cell lines from preimplantation blastocysts and their ability to develop into normal chimeras. These ES cells could spontaneously differentiate into cystic embryoid bodies with ectodermal, endodermal, and mesodermal cell types. Culture of these ES cells to confluence or chemical induction of differentiation resulted in morphological differentiation into fibroblasts, adipocytes, and epithelial, neuronal, and muscle cells. The differentiation of these embryonic cell lines into several cell types indicates a pluripotent ES cell. Furthermore, chimeric swine have been successfully produced using these ES cells. The hallmarks used by Wheeler were: spontaneous differentiation into embryoid bodies; their differentiation into several cell types; and their capacity to contribute to chimeras when combined with normal embryo cells.

Thomson et al., (1995) has reported the development of a primate ES cell line (Thomson JA, Kalishman J, Golos TG, Durning M, Harris CP, Becker RA, Hearn JP (1995) Isolation of a Primate Embryonic Stem Cell Line. *Proc. Natl. Acad. Sci. USA* **92**, 7844-8). The authors state: "When injected into severe combined immunodeficient mice, R278.5 cells consistently differentiate into derivatives of all three embryonic germ layers. **These results define R278.5 cells as an embryonic stem cell line...**" [Emphasis added]. R278.5 cells allowed to differentiate *in vitro* secrete certain distinct bioactive chemicals and express certain specific mRNAs indicating trophoblast and endoderm differentiation. Applicant submits that these hallmarks and definitions of ES cells would

be understood by persons of ordinary skill in the art and adequately supports the claims of the present invention pertaining to the use of ES cells.

The Examiner states on page 9 of the Office Action that "given the lack of guidance and unpredictability in obtaining ES cells . . . at the time of filing . . . the production of chimeric human/non-human animal chimera from ES cells is also not enabled." Yet numerous references disclosed development of embryos from ES cells. Non-human ES cells have been documented for the mouse (Martin, G. R. (1981) Isolation of a Pluripotent Cell Line from Early Mouse Embryos Cultured in Medium Conditioned by Teratocarcinoma Stem Cells. *Proc. Natl. Acad. Sci. USA* **78**, 7634-8), the pig (Wheeler (1994)), the rhesus monkey (Thomson et al. (1995)), and the marmoset, another primate (Thomson JA, Kalishman J, Golos TG, Durning M, Harris CP, Hearn JP (1996) Pluripotent Cell Lines Derived from Common Marmoset (*Callithrix jacchus*) Blastocysts, *Biol. Reprod.* **55**,254-9), prior to filing the application. The statement by the Examiner fails to account for the teachings of these references. Armed with the background provided by this work, undue experimentation would not be required to practice the embodiments of the claimed invention pertaining to the production of chimeras from human embryo cells and non-human ES cells (Claims 6, 7, 10-29, 33-36). The mouse embryonic stem cells of these studies were established directly from normal preimplantation mouse embryos. The embryonic stem cells are pluripotent and were isolated from inner cell masses of late blastocysts. Eight embryonic stem cell lines from these studies were derived from common marmoset blastocysts. These embryonic stem cell lines were shown to differentiate into a number of different cell types. These teachings would have made the degree of experimentation required reasonable.

With regard to the generation of human ES cells, although not widely published at the time the application was filed, many groups were using the techniques employed for other mammals to develop human ES cell lines. During the year following filing of the present application, two groups published the isolation of such cell lines (Thomson, J. A., Itskovitz-Eldor, J., Shapiro, S. S., Waknitz, M. A., Swiergiel, J. J., Marshall, V. S., and Jones, J. M. (1998) Embryonic Stem Cell Lines Derived from Human Blastocysts. *Science* **282,** 1145-7; and Shamblott, M. J., Axelman, J., Wang, S., Bugg, E. M., Littlefield, J. W., Donovan, P. J., Blumenthal, P. D., Huggins, G. R., and Gearhart, J. D. (1998) Derivation of Pluripotent Stem Cells from Cultured Human Primordial Germ Cells. *Proc. Natl. Acad. Sci. USA* **95,** 13726-31). These ES cell lines were isolated using existing techniques, without "undue experimentation." For example, in reporting the first primate ES cells Thomson et al. stated: "The growth of monkey ES cells in culture conditions that support feeder-dependent human EC [embryonal carcinoma] cells **suggests that similar conditions may support human ES cells**," (Thomson et al. (1995) at p. 7848; emphasis added). That this understanding was correct was confirmed in the report by this group of the isolation of human ES cells, where it is stated: "five [human] ES cell lines originating from five separate embryos were derived, **essentially as described for nonhuman primate ES cells**," (Thomson et al. (1998) at p. 1145; emphasis added). Applicant respectfully contends that the suggestion by the Examiner that existing art was insufficient at the time of filing to permit one of ordinary skill to characterize the properties and uses of anticipated human ES cells without undue experimentation is not correct.

IV. The Claims Satisfy 35 U.S.C. § 112, Second Paragraph

Claims 1-36 are rejected under 35 USC § 112, Second Paragraph, as being indefinite. This rejection is respectfully traversed. Applicant respectfully submits that the above-outlined new claims

and following remarks obviate the grounds for the rejection. Reconsideration and withdrawal of the rejection are respectfully requested.

The Examiner states that Claims 1-36 are "vague and indefinite" as to what would be considered a chimeric embryo. The Examiner cites page 1, lines 4-5 of the specification, as indicating that Applicant requires the chimeric embryos and animals to be "viable embryo forms." The Examiner has construed "forms" as a noun. This is a grammatical misreading of Applicant's specification, which referred to the embryo and ES cells being aggregated "under conditions in which a viable embryo forms," i.e., in which the formation of a viable embryo occurs. Applicant used "forms" in this case as a verb. Applicant clarifies that in the phrase "viable embryo **forms**," the word "forms" is used as a verb, and not a noun.

"Forms" does not indicate an "animal" or "animal form" that must exist. By "viable embryo," Applicant was referring to one which is alive (in the sense of respiration and not necessarily progressing full term) and capable of developing to the next or successive stage of development, as the term "viable embryo" is used in the developmental and reproductive biology literature (see Loi et al. (1999) and Behr et al. (1999), cited above, for examples). The fact that not all "viable" embryos would give rise to animals is not grounds for rejection of the claimed invention.

The Examiner further states that the Claims referring to "second animal species" are vague and indefinite. In part, this arises from the fact that "species" is both singular and plural. Applicant refers to "one or more second animal species" in Claim 1. In Claims 5-7, and 9, which refer back to Claim 1, "said second animal species" was also intended to include "one or more second animal species." Claim 1 is amended above to further read "wherein said second animal species is one or

more non-human mammals." For clarity, Applicant hereby amends Claims 5-7 and 9 to read "**from at least one of said second animal species**."

In Claim 19, Applicant refers to "one or more second animal species . . . wherein said second animal species is a non-human primate." For clarity, Applicant hereby amends Claim 19 to read " . . . wherein **at least one of** said second animal species is a non-human primate." For clarity, Applicant hereby also amends Claims 23-25, and Claim 27 to read " . . . from **at least one of** said second animal species . . ."

In Claim 28, Applicant refers to "one or more second animal species . . . wherein said second animal species is selected from among the group comprising chimpanzee, baboon, rhesus monkey, macaque, domestic pig, mouse, rat and rabbit." Applicant acknowledges that Applicant has used an improper form of Markush group. The term "**comprising**" was mistakenly used instead of "**consisting of**." Applicant's counsel apologizes for this error and any inconvenience it has caused and has amended the subject claims to correct this defect. In addition, Applicant amends Claims 32-34, and Claim 36, which refer back to Claim 28, to read " . . . from **at least one of** said second animal species . . ."

The Examiner states that Claims 13-15 are vague and indefinite because it is unclear what is meant by "developed from a chimeric embryo." "Developed from a chimeric embryo" has an unambiguous meaning in the scientific literature, which uses "develop" to refer to "embryonic development," or advancement to successive stage(s) and not to "reproduction." Applicant claims the progeny of chimeric animals as well, but only in Claims 16-18, which refer to a "decendent of said animal," not in Claims 13-15 as suggested by the Examiner. Applicant has amended the subject claims to further clarify the present invention.

The Examiner states that Claims 10-12 are vague and indefinite because it is unclear as to what would be required for a cell [sic] to be "developed" from a chimeric embryo. Applicant respectfully submits that it is referring to "cell lines," not cells. These cell lines are developed or generated from embryos by standard methods, known to one of ordinary skill in the art of developmental biology (e.g., Jakob, H. (1984) Stem Cells and Embryo-Derived Cell Lines: Tools for Study of Gene Expression. *Cell Differ.* **15,** 77-80; Shelton, J. N. (1990) Reproductive Technology in Animal Production. *Rev. Sci. Tech.* **9,** 825-45; Dushnik-Levinson, M., and Benvenisty, N. (1995) Embryogenesis *in vitro*: Study of Differentiation of Embryonic Stem Cells. *Biol. Neonate.* **67,** 77-83). For example, the cell lines of embryonic stem cells have been established in culture from blastocysts. For clarity, Applicant has amended Claim 10 to read, "A cell line **generated** from a chimeric embryo . . ."

The Examiner also questions how such a cell [sic] would be distinguishable from a cell [sic] prepared from any other source. Such a cell line is distinguished by the method of its preparation, which utilizes the present invention as part of the process. Cells of chimeras are known to differ in some respects from equivalent cells in non-chimeric animals, leading for example, to immunological tolerance of non-chimeric mothers to chimeras (Meinecke-Tillmann, S., and Meinecke, B. (1984) Experimental Chimaeras--Removal of Reproductive Barrier Between Sheep and Goat. *Nature* **307,** 637-8; Fehilly, C. B., Willadsen, S. M., and Tucker, E. M. (1984). Interspecific Chimaerism Between Sheep and Goat. *Nature* **307,** 634-6), of chimeras to grafted cells of the originating species (Gustafson, R. A., Anderson, G. B., BonDurant, R. H., and Mahi-Brown, C. (1993). Tolerance of Sheep-Goat Chimeras to their Component Cells. *J. Reprod. Immunol.* **23,** 155-68), and of reproductive efficacy of germ cells (Sumantri et al. (1997), see above). The cell lines of the

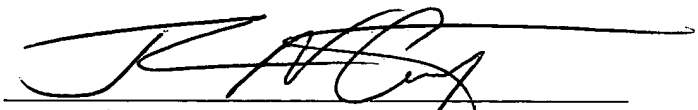
invention would, for example, permit the study of such immunological and reproductive properties at the cellular level.

IV. Conclusion

In view of the foregoing amendments and remarks, Applicant respectfully submits that the claims define statutory subject matter that is patentable over the art of record and the application is in condition for allowance. Should the Examiner believe anything further is desirable to place the application in better condition for allowance, the Examiner is invited to contact Applicant's undersigned attorney at the telephone number listed below.

Respectfully Submitted,

Date: June 16, 1999



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